Removal of *Escherichia coli* from biological effluents using natural and artificial mineral aggregates

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Abstract

Ability for disinfecting sterile biological effluents inoculated with *Escherichia coli* ATCC 25922 at concentrations of 10^5 CFU/mℓ, using a natural mineral aggregate (NMA) and artificial mineral aggregates (AMA's) consisting of individual oxides as Fe_2O_3, Cu_2O y Ag_2O and combined oxides as Fe_2O_3-Cu_2O, Fe_2O_3-Ag_2O, Cu_2O-Ag_2O, Fe_2O_3-Cu_2O-Ag_2O, contained in alginate beads, was compared. The results indicate that Ag_2O and Fe_2O_3-Ag_2O, Cu_2O-Ag_2O combinations, as well as NMA, inactivated 100% of *E. coli* in 30 min, whereas the oxides mixture, Fe_2O_3-Cu_2O-Ag_2O, took 13 min. It was observed that redox potential values were closely related to the disinfection level achieved. The advantage resulting from using alginate beads was that these allow the formation of AMA, which has higher disinfectant ability relative to NMA.

Keywords: disinfection, biological effluent, Fe_2O_3, Cu_2O and Ag_2O, alginate beads, *Escherichia coli*, natural mineral aggregate, artificial mineral aggregate

Introduction

Biological effluents from domestic wastewater treatment are required to be disinfected before reuse (Liberti et al., 2000) because they still contain microorganisms of intestinal origin, such as helminth ova and faecal coliform bacteria. *Escherichia coli* is a bacterium of enteric origin whose occurrence and abundance allows for its use in defining the sanitary quality of water and wastewater. The World Health Organization (WHO, 1989) has established a maximum level of 1 000 faecal coliforms unit (FCU)/100 mℓ for Category A water quality. Chlorination is the most widely used wastewater disinfection method, even though it has a drawback due to the formation of trihalomethanes and organochlorinated compounds which are carcinogens. An alternative disinfection method is the use of some metals, either alone or combined, such as Fe, Cu or Ag in the solid state (Davies and Etris, 1997; You et al., 2005), in ionic form (Craig, 2001; Jiang et al., 2006a; Silva-Martínez et al., 2004; Silvestry-Rodriguez et al., 2007), in combination with UV light (Kim et al., 2008) or as formulations where metal ions of Al, Cu or Ag are added to a solid matrix like zeolites (Rivera-Garza et al., 2000; De La Rosa-Gómez et al., 2008), ceramic material (Kim et al., 2004), silicates (Kawashita et al., 2003), colloids and metal nanoparticles (Chaloupka et al., 2010; Cho et al., 2005; Choi et al., 2008; Li et al., 2008), polymers (Lukhele et al., 2010) or biopolymers (Yi et al., 2003). However, experiences in using metals for disinfecting wastewater have been few, and mainly consist of using metal ions in combination with other chemical disinfectants, such as chlorine, hydrogen peroxide or peracetic acid (PAA). These combinations of disinfectants have been applied to influents from advanced primary treatment (APT), biological effluents or raw water (Pedahzur et al., 1995; Orta de Velásquez et al., 2008; Luna-Pabello et al., 2009). In most cases, for achieving total inactivation of test microorganisms, contact time tends to be large, i.e. up to 2 h (Table 1).

Mineral aggregates present an opportunity for improvement. Mineral aggregates may contain metals such as Fe, Cu and Ag at their different oxidation states, thus increasing their germicidal effect. These metals contribute to inhibition of the cellular respiration process, due to the inactivation of –SH radicals of respiration enzymes, the interruption of the electron-transfer chain and DNA and RNA disruption (Davies and Etris, 1997; Silva-Martínez et al., 2004; Holt and Bard, 2005; Sharma et al., 2005; Yamanaka et al., 2005; Silvestry-Rodriguez et al., 2007; Park et al., 2009; Chaloupka et al., 2010). Natural mineral aggregates (NMAs) have shown germicidal activity but they exhibit drawbacks such as not having a homogeneous composition and containing undesirable metals such as As and Pb (Miranda-Ríos and Luna-Pabello, 2002-2003).

A possible matrix to make artificial mineral aggregates is sodium alginate, which is a natural ionic polysaccharide having many applications in the food and pharmaceutical industries (Braccini and Pérez, 2001). Alginate has been used for immobilising biomolecules and also is a strong chelating agent for metals. With most divalent cations, it produces gels that are heat irreversible (Park et al., 2007).

Based on the above, the objective of this study was to determine the contact time required to disinfect a biological effluent containing *E. coli*, at initial concentrations of 10^5 CFU/mℓ, using silver shot, copper shot, natural mineral aggregates (NMA's) and artificial mineral aggregates (AMA's) formed with Fe_2O_3, Cu_2O, Ag_2O, separated or combined.

Experimental

Sterile biological effluent: The biological effluent was obtained from Ciudad Universitaria UNAM, located at the southern zone of Mexico City. Two hundred litres were collected from the effluent of the activated sludge system, before it passes through the sand filter, and was subjected to physicochemical analysis as described by Eaton et al. (2005), and then sterilised by autoclaving at 1.1 kg/cm^2, 120ºC for 15 min.

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Received 16 January 2010; accepted in revised form 1 February 2011.

Table 1
Water and wastewater test disinfection using metals

Disinfectant/ Concentration	Test water	Test microorganism/ Concentration	Contact time/ Inactivation	Reference
Zerovalent Fe in granules or shot 1.0 g	Artificial groundwater	Bacteriophages: 1) *F* X174 2) MS-2 1×10^5 plaque-forming unit/mℓ	120 min 1) 79.41%, 0.7 $\log_{10}$ 2) 94.94%, 1.3 $\log_{10}$	You et al., 2005
Zerovalent Ag in granules or shot 1.0 g	Sterile biological secondary effluent	*Escherichia coli* ATCC 25922, 7.4×10^5 CFU/ 100 mℓ	90 min, 100%, 3 $\log_{10}$	Miranda-Ríos and Luna-Pabello, 2002-2003
K_2FeO_4 Fe (VI), 1) 6.0 mg/ℓ 2) a. 6.0 mg/ℓ b. 15.0 mg/ℓ	1) Sterile phosphate buffered water 2) Secondary sewage effluent	f2 virus 1×10^5 -1×10^7 cells/mℓ	1) 1 min, 99% f2 virus 2) a. 13 min, 99% for f2 virus b. 60 min, 99% for bacteria, 1 min, 100% for f2 virus	Schink and Waite, 1980
K_2FeO_4 Fe (VI) 1) 6.0 mg/ℓ 2) 15.0 mg/ℓ	1) Tap water 2) Raw wastewater	1) *E. coli* 3.2×10^8 CFU/ 100 mℓ 2) Faecal coliforms 3.3×10^8 - 2×10^9 FCU/100mℓ	1) 30 min, 100%, 8 $\log_{10}$ 2) 80 min, 99.99%, > 4 $\log_{10}$	Jiang et al., 2006a; b; 2007
Electrolytic Cu and Ag ions 1) 1.2 mg/ℓ Cu/ 0.6 mg/ℓ Ag 2) 0.6 mg/ℓ Cu/ 1.2 mg/ℓ Ag 3) 1.2 mg/ℓ Cu/ 0.2 mg/ℓ Ag / 0.3 mg/ℓ Cl_2	1) Biological secondary effluent 2) Cooling water	1) Total coliforms and *E. coli,* 2.5×10^8 MPN/100 mℓ 2) Total coliforms, 5.0×10^8 MPN/100 mℓ and *E. coli,* 2.0×10^8 MPN/100 mℓ	1) 2.0 h, total elimination of coliforms and *E. coli* 2) 2.0 h, total elimination of coliforms and *E. coli*	Silva-Martínez et al., 2004
Cu, Ag ions and Cl_2 1) 0.8 mg/ℓ Cu, 0.08 mg/ℓ Ag 2) 0.8 mg/ℓ Cu/0.08 mg/ℓ Ag/1.00 mg/ℓ Cl	Well water	*Naeglaeria fowleri* ATCC 30894, 1×10^4 viable amoebas/ mℓ	1) 72 h, 14.5% (0.58 $\log_{10}$) 2) 3.9 min, 99%	Cassells et al., 1995
Ag, Cu 1) 100 +1 000µg/ℓ 2) 500 +5 000µg/ℓ	*In vitro*	*Hartmannella vermiformis* amoebas and the ciliated protozoan *Tetrahymena pyriformis*	1)*Tetrahymena* and *Hartmannella,* 2 log reduction 2) *Hartmannella,* 0.6 log reduction.	Rohr et al., 2000
Fe(+3) or Cu (+2) in zeolite (27.5 and 2.0 mg/g)	Municipal sewage treatment plant	1) Faecal coliforms	1) 2 log reduction in 6 h	Milan et al., 2001
Ag zeolite (14% w/w)	Residual efluent	10^6 FCU/100 mℓ	110-129 min, Category A	De la Rosa-Gómez et al., 2008
1) $AgNO_3$ (1.0 mg/ℓ) 2) Ag ions and UV-A (54 mW/cm2, 300-400 nm) 3) Ag ions and visible light irradiation (93 mW/cm2, 400-700 nm)	Phosphate buffer pH=7	*E. coli* and MS-2 phage 1×10^5-2×10^5 CFU or PFU/mℓ	1) 30 min, 1.5 log *E. coli* and 2.5 log MS-2 phage 2) 30 min, 4.5 log *E. coli* and 5.0 log MS-2 phage 3) 30 min, 2.0 log *E. coli* and 4.5 log MS-2 phage	Kim et al., 2008
1) Colloidal Ag nanoparticles (20 µg/g and 10 µg/g) 2) Colloidal Pt nanoparticles (20 µg/g and 10 µg/g)	LB medium diluted in NaCl (0.85%)	a) *Staphylococcus aureus* b) *E. coli* 10^5- 10^6 CFU/mℓ	1) a. 3.3-4 h total elimination b. 2.5 to 3.5 h. total elimination 2) No elimination	Cho et al., 2005
1) Ag nanoparticles 2) Silver ions (AgCl) 3) AgCl colloidal 1.4 µM 2.8 µM 4.2 µM	BBL broth	*E. coli* PHL628-gfp (No data)	At 5 hours 1) a. 17.0% b. 30.0% c. 55.0% 2) a. 11.0% b. 69.0% c. 100.0% 3) a. 7.0% b. 24.0% c. 66.0%	Choi et al., 2008

Available on website http://www.wrc.org.za
ISSN 0378-4738 (Print) = Water SA Vol. 37 No. 2 April 2011
ISSN 1816-7950 (On-line) = Water SA Vol. 37 No. 2 April 2011

Silver nanoparticles in polymeric microspheres, 100 mg	Autoclaved water	*E. coli* ATCC 8739 (7×10^6 CFU/mℓ) *P. aeruginosa* ATCC 9027 (22×10^6 CFU/mℓ) *B. subtilis* ATCC 6051 (46×10^6 CFU/mℓ) *S. aureus* ATCC 25923 (24×10^6 CFU/mℓ)	At 2 h 2.64 log 3.87 log 4.06 log 2.65 log	Gangadharan et al., 2010
Silver ($AgNO_3$ 0.01M) carbon nanotubes polymerised with β cyclodextrin Carbon nanotubes polymerized with β cyclodextrin Polyurethane with β cyclodextrin	Sterile distilled water	*E.coli* (ATCC25925) (1.3×10^7 CFU/mℓ)	30 min, 94% 60 min, 95% 90 min, 100% 30 min, 84% 60 min, 48% 90 min, 45% 30 min, 72% 60 min, 70% 90 min, 48%	Lukhele et al., 2010
1) PAA and Ag (7.5-1.0 mg/ℓ) 2) H_2O_2 and Ag (200-1.0 mg/ℓ) 3) H_2O_2, Cu (50-1.0 mg/ℓ)	APT effluent	10^6 FCU/100 mℓ	1) 45 min Category A 2) 30 min Category A 3) 30 min Category A; 120 min total elimination	Orta de Velásquez et al., 2008
PAA, Cu, Ag (20.0-0.1-1.0 mg/ℓ)	Biological effluent	10^5 FCU/100 mℓ CF	10 min Category A	Luna-Pabello et al., 2009
Colloidal silver (0.5 mg/ℓ)	Sterilised biological efluent	10^6 CFU/100 mℓ *E. coli*	15 min total elimination	Miranda-Ríos and Luna-Pabello, 2002-2003

Inoculum: *Escherichia coli* ATCC 25922 bacteria preserved on nutritive agar (BBL) were inoculated in an Erlenmeyer flask containing 100.0 mℓ of sterile nutritive broth (BBL) and were placed overnight in an incubator with orbital agitation (G24 New Brunswick) at 37ºC and 250.0 r/min. After 18 h of incubation the inoculum was adjusted with nutritive broth at OD of 1.4 at 600 nm wavelength using an UV-VIS spectrophotometer (Pharmacia Biotech, Ultrospec 3000). Subsequently, 10.0 mℓ of inoculum were diluted in 99.0 mℓ of sterile distilled water. One millilitre from this decimal dilution was pre-adapted in a flask containing 99.0 mℓ of the sterile biological effluent. The flask was again placed in the incubator with orbital agitation for 24 h.

Escherichia coli presence is the most reliable indicator of faecal bacterial contamination of surface waters in different countries. An appropriate health-based indicator of microbial pathogens should possess several characteristics (Arana et al., 2000). The indicator should always be present when pathogens are present and should not be detected when the pathogens are absent; it should have a life span similar to that of the pathogens of concern; it should be present in large numbers and should be readily detected by simple and inexpensive methods; and it should not multiply in the environment once it has been shed by the host. Based on these conditions, if the indicator is isolated from the water under examination the pathogenic organisms could still be present; if the indicator is absent, pathogenic organisms are also probably absent (Eaton et al., 2005; Kim et al., 2008).

Water for testing: Water for testing was prepared by adding 5.0 mℓ of pre-adapted *E. coli* at a concentration of 10^6 CFU/mℓ for each 95.0 mℓ of sterile biological effluent, to obtain a concentration of 10^5 CFU/mℓ.

Preparation of alginate beads: Sodium alginate solution was prepared dissolving 7.5 g of sodium alginate (Sigma), 3 500 mPa·s and 2.5 g sodium alginate (Sigma), 14 000 mPa·s, in 400 mℓ of distilled water. This solution was sterilised by autoclaving, was allowed to cool and then powdered Fe_2O_3, Cu_2O (J.T. Baker) and Ag_2O (Merck) were added to it. The solution was homogenised with a magnetic agitator until it was completely dissolved. The addition of each oxide was calculated for obtaining the same percentage by weight as that of NMA (see Table 2). The above mixture was taken by a 3.0 mℓ sterile syringe and then was added drop by drop to a 2.0% calcium chloride solution (J.T. Baker), thus forming alginate beads which contain metals. These beads, which have a diameter of 2.00 mm, are filtered off from the $CaCl_2$ solution and are allowed to dry. As a blank, alginate beads with no added metals were prepared.

Table 2
Metal oxides content in beads formed with sodium alginate

Oxide in the alginate beads	Sodium alginate (g)	Metals (g)		
		Fe_2O_3	Cu_2O	Ag_2O
Fe_2O_3	97.46	2.5449	-	-
Cu_2O	99.99	-	0.0116	-
Ag_2O	99.99	-	-	0.0109
Fe_2O_3-Cu_2O	99.44	2.5449	0.0116	-
Fe_2O_3-Ag_2O	99.44	2.5449	-	0.0109
Cu_2O-Ag_2O	99.98	-	0.0116	0.0109
Fe_2O_3-Cu_2O-Ag_2O	97.43	2.5449	0.0116	0.0109

Microbiological analysis: The concentration of *E. coli* was determined by the dilutions method and by the technique of membrane filter using sterile nitrocellulose filters (Millipore,

Bedford, MA, USA) with a pore size of 0.45 µm and a diameter of 47 mm and agar M-FC (BBL) added with rosolic acid (Hycel de México, S.A. de C.V.) at 2% in NaOH (Merck) (Eaton et al., 2005). Petri dishes were placed in a water jacketed incubator (Ac-Lab) for 24 h at a temperature of 44.5°C.

Physicochemical characterisation of NMA: A natural mineral aggregate (NMA) with a particle size ranging between 2.0 to 3.36 nm, was obtained from a mine located in Zacatecas State, Mexico. This material was characterised by X-ray diffraction techniques and inductively-coupled **plasma atomic emission spectroscopy (**ICP-OES**) (**Eaton et al., 2005**).**

Disinfection tests: To evaluate disinfecting capacity of NMA and alginate beads containing separate oxides, 4 tests were carried out:

- NMA
- Alginate beads containing separate oxides
- Oxide pairs: Fe_2O_3-Cu_2O, Fe_2O_3-Ag_2O and Cu_2O-Ag_2O
- Triple combination Fe_2O_3-Cu_2O-Ag_2O

From each flask, 100.0 mℓ of water for testing was taken, and for each test either 1 g of NMA or 1 g of alginate beads was added, as shown in Table 3. Flasks were placed in an incubator with orbital agitation, at 37°C and 250 r/min. At pre-established contact times of 0.0, 30.0 and 60.0 min or 0, 15.0 and 30.0 min, the concentration of surviving bacteria was determined, as well as pH, dissolved O_2 and redox potential. The disinfectant activity of alginate beads containing metal oxides and NMA was stopped by adding a neutralising solution prepared with 280.0 mℓ of Tween 80 (Sigma), 40.0 g of soy lecithin and 1.25 mℓ of phosphate buffer solution, making the volume up to 1 ℓ with distilled water. Once homogenised, the solution was sterilised by autoclaving at 1.1 kg/cm^2, 120ºC for 15 min (Bloomfield, 1991). This neutralising solution was used either applying 1.0 mℓ on the membrane, where direct seeding is carried out, or in amounts of 9.0 mℓ applied into the assay tubes used for preparing the 10^{-1} decimal dilution, for seeding by diluting. The experiments were conducted in triplicate and the results presented are mean values. The results were analysed statistically using SPSS 15.0 software. Statistical analysis consisting of a factorial analysis of variance (ANOVA) was performed on the entire data set to determine if significant differences existed between the results obtained using different types of disinfectant (NMA or AMA) and contact time. The differences between treatments were analysed by Tukey´s HSD (Honestly Significantly Different) test at $P<0.05$.

Kinetics of disinfection – The kinetics of disinfection was established according to the Hom equation (Gyürék and Finch, 1998). In this model, the concentration of disinfectant remains unchanged during the disinfection process.

$$N_t / N_0 = \exp(-k^* t^m) \quad \text{(i)}$$

where:

t = time (minutes)

Nt/N0 = quantity of surviving microorganisms,

k* =Constant, time -1, this constant includes the die-off coefficient and the disinfectant doses C^n (when n=1) and m without change.

Table 3
Experimental conditions for disinfection test

Test	Disinfectant (1 g)	Contact time (min)
NMA	NMA	0,15,30,60
Metal	Alginate beads containing Fe_2O_3, Alginate beads containing Cu_2O Alginate beads containing Ag_2O	0,15,30,60
Metal pairs	Alginate beads containing Fe_2O_3 and Cu_2O Alginate beads containing Fe_2O_3 and Ag_2O Alginate beads containing Cu_2O and Ag_2O	0,15,30
Combination of 3 metals	Alginate beads containing Fe_2O_3, Cu_2O and Ag_2O	0,5,10,15 and 0,15,30

Table 4
Microbiological and physicochemical characteristics of the biological effluent before it was subjected to sterilisation

Parámeter		Parámeter	
pH (25°C)	7.7	Arsenic (mg/ℓ)	0.0007
BOD_5 (mg/ℓ)	32	Cadmium (mg/ℓ)	<0.005
COD (mg/ℓ)	99	Cyanides (mg/ℓ)	<0.02
TDS (mg/ℓ)	18	Copper (mg/ℓ)	0.01
SS (mℓ/ℓ)	<0.1	Chromium (mg/ℓ)	<0.02
Nitrates (mg/ℓ)	1.86	Mercury (mg/ℓ)	0.0015
Nitrites (mg/ℓ)	0.097	Nickel (mg/ℓ)	<0.025
Kjeldahl Nitrogen (mg/ℓ)	22.85	Silver (mg/ℓ)	<0.01
Total nitrogen (mg/ℓ)	24.807	Lead (mg/ℓ)	<0.025
Total phosphorus (mg/ℓ)	4.14	Zinc (mg/ℓ)	<0.01
Oil and greases (mg/ℓ)	<5		
Faecal coliforms (FCU/mℓ)	$8.6x10^3$	Helminth ova (HE/ℓ)	4-5

Results and discussion

The results of microbiological and physicochemical characterisation of the effluent of the activated sludge system before it was sterilised, are shown in Table 4. The data indicate that it corresponds to a typical secondary effluent (Metcalf and Eddy, 2004; Orta de Velásquez et al., 2008; Luna-Pabello et al., 2009).

The results of physicochemical characterisation of NMA by the x-ray diffraction technique indicate that is comprised of quartz, sanidine, nymite, montmorillonite, calcite and Fe oxide (III). ICP-OES analysis indicated that NMA contains Fe (1.78 % w/w) in a higher proportion than other metals, such as Cu (0.0103% w/w), Zn (0.0743% w/w), As (0.0037 %w/w), Ag (0.0101% w/w) and Pb (0.0448% w/w). Despite the concentrations of As and Pb being detected in low proportions, it is desirable that they would not be present in a disinfected effluent.

X-ray analysis of the bulk flotation product indicated that pyrite (FeS_2) is the main form of Fe and indicated the presence of calcite ($CaCO_3$) in the NMA. The calcite in the NMA would neutralise any H_2SO_4 that might form from a possible pyrite oxidation, freeing carbonate anions. Also, as a result of

Available on website http://www.wrc.org.za
ISSN 0378-4738 (Print) = Water SA Vol. 37 No. 2 April 2011
ISSN 1816-7950 (On-line) = Water SA Vol. 37 No. 2 April 2011

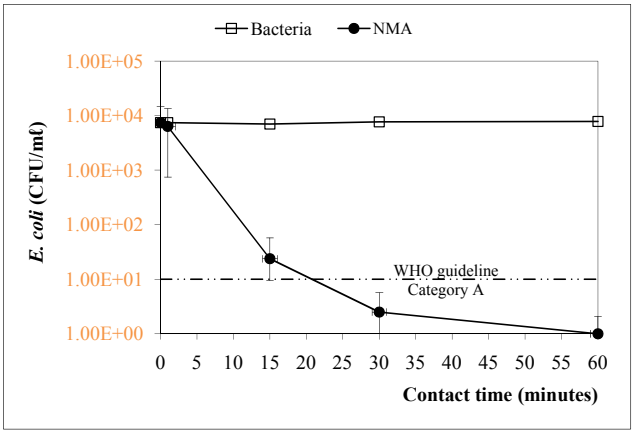

Figure 1
Inactivation of E. coli *using NMA*

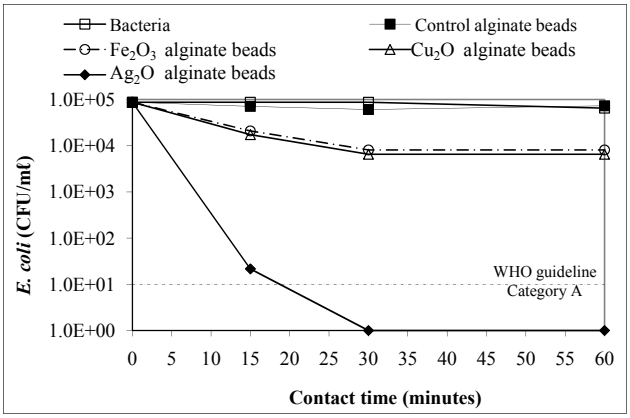

Figure 2
Inactivation of E. coli *by alginate beads containing separated metal oxides*

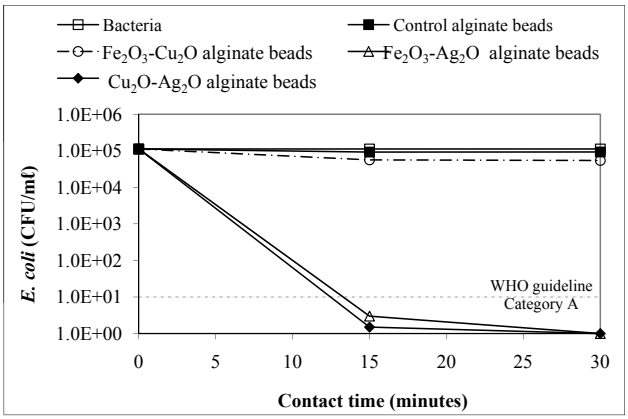

Figure 3
Inactivation of E. coli *by alginate beads containing metal oxide pairs*

prevalent acidity conditions, soluble Pb and As species would form their carbonates and would adsorb onto cemented layers or precipitate on the calcite, and thus reduce the aqueous availability of these metals (Armienta and González Hernández, 2007; Smedley, 2008; Romero et al., 2008; Espinosa et al., 2009).

In the NMA experiments, redox potential in test water was oxidant (132.6±0.85 mV). Since the pH values were around 8.5 ±0.03 units, As and Pb could not be incorporated into the final effluent.. After 30 min of contact between test water and NMA, Fe, Cu, As, Ag and Pb concentrations were lower than the detection limit in the test water, and Zn had a concentration of 0.58 mg/ℓ.

The tests conducted with NMA indicated that almost 100% of 10^5 CFU/mℓ *E. coli* may be removed during 30 min of contact (Fig. 1), whereas with copper shot and silver shot 90 min are needed (Miranda-Ríos and Luna-Pabello, 2002-2003). NMA requires from 15 to 30 min to obtain an effluent that would be considered by WHO as Category A, that is, suitable to for reuse in agricultural irrigation (less than 1 000 FCU/100 mℓ), while a zeolite containing silver at a concentration of 1.4% w/w needs 110 min to achieve the same disinfection level (De La Rosa-Gómez et al., 2008). Consequently, the combination of metals in NMA exerts a synergistic effect on disinfection because it requires 80 min less than silver zeolite and 60 min less than either copper shot or silver shot to achieve the same results. A Tukey HSD test (P<0.05) showed statistically significant differences between the disinfection % at 15 min of contact time achieved with use of NMA (99.679%) and AMA with Ag (i.e. Ag_2O (99.990%), Fe_2O_3-Ag_2O (99.997%), Cu_2O-Ag_2O (99.999%) and Fe_2O_3-Cu_2O-Ag_2O (100.000%) alginate beads) versus AMA without Ag (i.e. Fe_2O_3 (76.010%), Cu_2O (79.940%) and Fe_2O_3-Cu_2O (49.820%) alginate beads).

In Fig. 2 and Fig. 3 it can be seen that both alginate beads containing Ag_2O and those formed with Fe_2O_3-Ag_2O or Cu_2O-Ag_2O combinations require less than 30 min contact time to achieve the total removal of bacteria. In a similar time period, beads with Fe_2O_3 and those containing Cu_2O reduce the initial content of *E. coli* by less than 1 base-10 logarithmic unit (log_{10}). Moreover, after a contact time of 15 min, beads with Fe_2O_3-Cu_2O combination reduce the content of *E. coli* only by 0.32 log_{10}, whereas with beads containing Fe_2O_3-Ag_2O o Cu_2O-Ag_2O the concentration decreases 0.48 and 0.17 log_{10} units, respectively. Finally, for the beads containing Ag_2O, the concentration was reduced to 1.33 log_{10} units, resulting in a survival of more than 1 log_{10}.

The value corresponding to a Category A effluent is achieved at a contact time from 15 to 30 min for Ag_2O beads and from 10 to 15 min for Fe_2O_3-Ag_2O and Cu_2O-Ag_2O beads. As shown in Figs. 2 and 3, the effect produced by the union between Ag_2O and Fe_2O_3 or Cu_2O is an increase in the disinfecting capacity of silver oxide, requiring a shorter contact time to remove the *E. coli* added, due to the synergistic effect of these oxides on each other. Similar observations for an increase in disinfecting capacity were reported for the union of electrolytically-produced Cu and Ag ions (Rohr et al., 2000; Silva-Martínez et al., 2004) or Cu and Ag oxides contained in a ceramic matrix (Kim et al., 2004), for water from cooling towers; however, a synergistic effect on disinfection due to Fe_2O_3 and Ag_2O has not been reported.

Figure 4 shows that alginate beads containing the 3 oxides, Fe_2O_3, Cu_2O and Ag_2O, require a contact time of 13 min to completely remove *E. coli*, and from 5 to 10 min to reach the values established by WHO for a Category A effluent. The increase in removal of *E. coli* is again attributed to the synergism of the 3 metals. As observed in Fig. 3, the disinfecting capacity of the Fe_2O_3-Cu_2O combination is minimal; therefore the increase in the disinfecting capacity with the combination of the 3 oxides is not only attributable to the metals involved, but to the presence of oxygen in the medium. In this sense, an increase in bacterial inactivation has been demonstrated by the production of oxygen-reactive species by silver (Davies and Etris, 1997), both in the matrix containing it (Inoue et al., 2002)

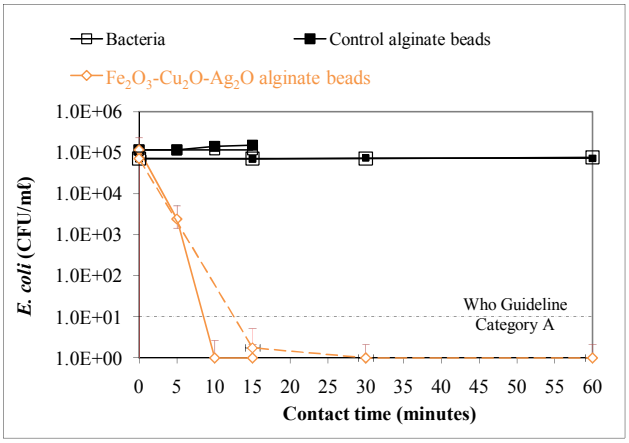

Figure 4
Inactivation of E. coli by alginate beads containing 3 metal oxides
0, 15 and 30 min (----) and 0, 5, 10 and 15 min (——)

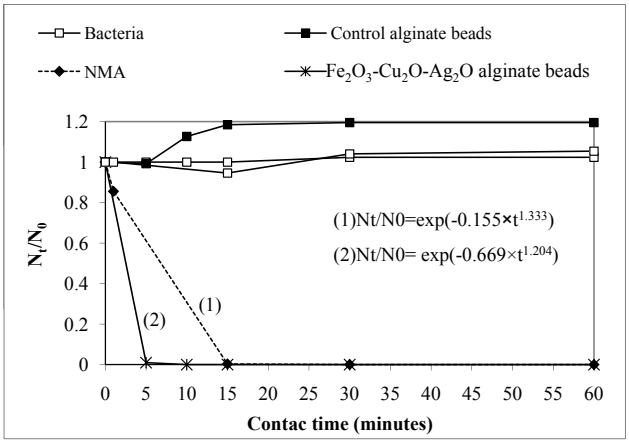

Figure 5
Disinfecting effect of alginate beads simultaneously containing the 3 metal oxides and that obtained with NMA

or by intracellular production as a consequence of the damage that silver causes by forming insoluble complexes with DNA and cellular RNA (Park et al., 2009). This causes the interruption of growth, metabolism and reproduction in the affected cell, as well as changes in structure and permeability of the cell membrane, disturbing the interchange of material between cell and its environment. Silver causes the cytoplasmic membrane to shrink and to separate from the cell wall (Feng et al., 2000).

An increase in the bacterial inactivation effect produced by disinfectants generating reactive oxygen species like H_2O_2 and PAA, and Cu and Ag in their ionic states, was observed for APT and biological treatment effluents (Orta de Velásquez et al., 2008; Luna-Pabello et al., 2009). In these experiments, the time required to obtain Category A effluents was 30 min for H_2O_2-Cu (50.0-1.0 mg/ℓ), H_2O_2-Ag (200.0-1.0 mg/ℓ) or PAA-Ag (7.5-1.0 mg/ℓ) combinations and was reduced by 10 min for Cu-Ag-PAA (0.1-1.0 -20.0 mg/ℓ) combination. That is, these contact times were higher than those required for the combination of the 3 metallic oxides. This supports the theory that oxygen contained in the oxides of the 3 metals used plays an active role in disinfection.

In Fig. 5, an increase in disinfection by using the combination of the 3 metals is observed, where a contact time lower than 5 min. was needed for removing 10^5 CFU/mℓ *E. coli*, while NMA requires 30 min (Fig. 1). Consequently, combination of the 3 metals is a good option in disinfection of effluents from biological treatment systems, without the presence of undesirable metals such as Pb and As (Miranda-Ríos and Luna-Pabello, 2002-2003).

NMA required a contact time of 30 min (Fig. 1) and AMA required 13 min (Fig. 4). The *E. coli* inactivation kinetics followed the Hom equation using either the NMA or the Fe_2O_3-Cu_2O-Ag_2O AMA and the corresponding equations were: $Nt/N0= \exp(-0.1555 \times t^{1.3327})$ and $Nt/N0= \exp(-0.669 \times t^{1.204})$, respectively. In both cases, it was observed that the inactivation rate increases with the contact time due to the fact that *m* is greater than 1.0. The rate constant is more than 2 times greater for the AMA than for the NMA, according to its greater disinfectant power.

Also, the NMA rate constant is very close to that measured for the Cu-Ag PAA (k*=-0.1612) (Luna-Pabello et al., 2009). In both cases, the silver concentration was 1.0 mg/ℓ and the initial bacteria concentration was 10^5 CFU/100 mℓ.

In the case of the use of chloramine, at a concentration level of 2.4 mg/ℓ, to disinfect a biological effluent, a rate constant of -0.361 was measured, which is close to that found for the Fe_2O_3-Cu_2O-Ag_2O beads. However, the *m* exponent for chloramine disinfection (m=0.715) is lower than 1.0 (Pretorius and Pretorius, 1999); that is, the rate decreases with the contact time, which implies that chloramine is in fact consumed during the disinfection. This is in contrast to disinfection with Fe_2O_3-Cu_2O-Ag_2O beads, for which no metal consumption has been observed.

Moreover, for all tests performed, the pH was maintained at about 8.5, whereas in the case of dissolved oxygen there was a slight tendency to decrease from 5.6 to 5.1 mg/ℓ O_2. With regard to redox potential, the value increased by 18.0 mV in assays where Ag_2O y Fe_2O_3-Ag_2O was used; by 20 mV for Cu_2O-Ag_2O beads; by 34 mV for Fe_2O_3-Cu_2O-Ag_2O beads; and by 24 mV for NMA. This increase in redox potential, where the environment becomes more oxidising, can be related to *E. coli* removal, as a greater increase produces a smaller contact time to inactivate the bacteria. For flasks containing alginate beads comprising Cu_2O, Fe_3O_2 and Fe_2O_3-Cu_2O combination, redox potential was maintained about 8 mV. These beads showed very low disinfecting capacity (less than 1 base-10 logarithmic unit).

In Mexico, wastewater is a valuable (and sometimes the only) resource available for crop irrigation, but is frequently reused in agriculture without any proper application of disinfection measures. This inevitably poses a grave risk to health. Therefore, the importance of this type of study lies in the priority it attaches to treating the high levels of microbial contamination which exist in wastewater, when said wastewater is destined for reuse in agriculture (Orta de Velásquez et al., 2008).

Chlorination is the wastewater disinfection method more widely used, even though it might lead to the formation of trihalomethanes and organochlorinated compounds which are carcinogens. The main alternatives to chlorination are ozonation, and the use of ultraviolet light. According to estimates carried out by Collivignarelli et al. (2000), the investment cost for the disinfection of wastewater previously treated by a biological system varies, depending on the size of the plant, as follows (in South African Rands): Chlorine dioxide = ZAR138 427 to 1 993 343; ozone = ZAR346 067 to 5 613 198; ultraviolet light = ZAR263 010 to 7 336 609. Another possible

Available on website http://www.wrc.org.za
ISSN 0378-4738 (Print) = Water SA Vol. 37 No. 2 April 2011
ISSN 1816-7950 (On-line) = Water SA Vol. 37 No. 2 April 2011

technical alternative for the disinfection of raw or partially treated wastewater is the use of metals such as silver (Ag) and copper (Cu). However, there is little information available on this subject.

It should be noted that as the wastewater moves forward in the treatment train its faecal coliform content as well as its nutritional content diminishes. For this reason, if the adequate disinfection of wastewater at the early stages of the treatment is achieved using low concentrations of metals, it would be possible to preserve the nutrients and this would represent an advantage when used in agricultural irrigation, while avoiding the creation of carcinogenic compounds associated with the addition of chlorine (Keraita et al., 2008; Luna-Pabello et al., 2009). Before widespread application can be recommended, however, economic feasibility studies need to be conducted. Nevertheless, the alternative remains potentially interesting for developing countries.

Conclusions

In the case of NMA, for alginate beads containing Ag_2O and combinations of Fe_2O_3-Ag_2O and Cu_2O-Ag_2O, 30 min of contact time were required for inactivating 100% of *E. coli* at a concentration of 10^5 CFU/mℓ. For beads containing the Fe_2O_3-Cu_2O-Ag_2O mixture 13 min were required. In order to attain Category A water quality, a contact time of 15 to 30 min was required for NMA and Ag_2O beads, whereas 10 to 15 min was required for Fe_2O_3-Ag_2O y Cu_2O-Ag_2O beads. It was observed that redox potential values are closely related to the disinfection level achieved. The need to use less time to achieve the desired disinfection level is closely related to the synergistic effect of the metals present. The observed sequence of decreasing bacterial inactivation effect was as follows: Fe_2O_3-Cu_2O-Ag_2O>Fe_2O_3-Ag_2O=Cu_2O-Ag_2O>Ag_2O=NMA>Fe_2O_3=Cu_2O=Fe_2O_3-Cu_2O. The advantage of using alginate beads is that it allows the formation of AMA, which has a greater disinfecting capacity than NMA.

Acknowledgements

The authors acknowledge the financial support of the DGAPA (UNAM) through PAPIIT Project IN215006 and IN107209. Graduate scholarship for MMR was provided by CONACYT. The authors thank the anonymous reviewers for their valuable suggestions that greatly improved the manuscript.

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Available on website http://www.wrc.org.za
ISSN 0378-4738 (Print) = Water SA Vol. 37 No. 2 April 2011
ISSN 1816-7950 (On-line) = Water SA Vol. 37 No. 2 April 2011